

Mexican Wolf Blue Range Reintroduction Project

Adaptive Management Oversight Committee

Standard Operating Procedure

Title: Blood Collection, Handling, and Storage

Number: 23.0

File Name: MW SOP 23.Blood Collection.Final.20050430.doc

Purpose: This SOP provides guidelines for safe collection, handling, and storage of blood samples from live Mexican gray wolves. It supersedes relevant sections of the 1998 Mexican Wolf Interagency Management Plan (USFWS 1998), and therefore represents, in part, the “Service Approved Management Plan” referenced in the Mexican Wolf Final Rule (50 CFR 17.84(k)).

Exceptions: None. Per SOP 2.0, AMOC must approve any exceptions to this SOP.

Background: Blood collection is important to the Project in order to address and monitor trends in wolf population health, disease, and genetics. These guidelines apply to all Mexican wolves and other canids (i.e. feral dogs, coyotes, etc.) that are captured in the wild. Standard procedure for the Project is to collect blood for genetic testing from any Mexican wolf captured in the wild. The purpose of the testing is to determine if the animal is a Mexican wolf and further, to determine the parentage of the animal, thereby identifying its pack affiliation. Blood is also collected and banked from any non-target canids (feral dogs, coyotes, etc.) that are captured in traps set for Mexican wolves in order to monitor for possible introgression of coyote or dog genes into the free-ranging Mexican wolf population. Project staff must make every effort to ensure that samples are of good quality and adequate for all necessary testing, while minimizing handling stress to the animal and maintaining safety of the personnel involved. Because blood collection, handling, and banking procedures may vary at the pre-release captive wolf facilities, the procedures described in this SOP apply only to obtaining blood from free-ranging canids in the BRWRA.

Procedures:

1. Blood Collection
 - a. Equipment and Supplies
 - i. Animal that blood is to be drawn from. Blood must be taken from all wolves captured in the wild. This is especially important for uncollared, wild-born wolves in order to determine parentage and monitoring of genetics. Blood collection from other wolves (known wolves or collared wolves in which blood is already banked) is less important but should be taken to monitor for individual health, exposure to disease, etc. Blood should also be taken from any other non-target canid such as dogs or coyotes that are captured in order to monitor for potential introgression into the Mexican wolf population.
 - ii. Sterile syringes or vacutainers and needles (20 gauge or smaller gauge = larger diameter).
 - iii. Tourniquet with quick release.
 - iv. Blood tubes.

- (1) Two red tops – 7ml.
 - (2) Two purple tops – 7ml.
 - v. Permanent pen (Sharpie) to label blood tubes.
 - vi. Sharps safety disposal (for used syringes and needles).
 - vii. Cooler with ice pack wrapped in towel.
 - b. Selecting a vein or artery.
 - i. This is up to the individual's preference.
 - ii. Common collection sites are the cephalic, saphenous, or the jugular veins.
 - c. Drawing blood
 - i. Personnel should have experience and a certain amount of proficiency when attempting blood collection. Project personnel are provided ample opportunities to practice blood drawing at numerous captures held at the Sevilleta and Ladder Ranch wolf facilities and are encouraged to attend to perfect their blood drawing skills.
 - ii. The animal must be adequately immobilized or restrained for blood collection to reduce blood collection time, stress on the animal, and risk of injury to personnel.
 - iii. Draw blood. See Appendix A (Blood Collection from Wild Canids) for additional guidance on blood collecting.
 - iv. Minimum amount needed.
 - v. Two 7 ml red tops per wolf; no red tops are needed for dogs or coyotes.
 - vi. Two 7 ml purple tops per wolf ; one purple top for dogs or coyotes.
 - vii. Label blood tubes with species, studbook ID number (only if wolf), and date.
 - viii. Place samples in cooler; avoid placing blood tubes in direct contact with ice packs.
2. Blood Handling
- a. Processing Blood:
 - i. Red tops (collected on wolves only; not needed for coyotes or dogs) – for blood chemistry.
 - (1) Spin down red tops in centrifuge for 10 minutes. Centrifuges are located at the Alpine Field Office and the Sevilleta veterinary trailer.
 - (2) Draw out the serum without agitating the red blood cells using a pipette.
 - (3) Put the serum into a cryovial and label with species, wolf's studbook number, date, and "serum." Example: Mexican wolf, SB #511, 10/31/04, Serum.
 - (4) Freeze cryovials until they are ready to ship.
 - (5) The remaining red blood cells can be drawn out of the tubes and frozen in cryovials to bank. Label as described above substituting "red blood cells" for "serum."
 - (a) This sample can be banked for later genetics work.
 - ii. Purple tops (used for genetics, CBC, and banking).
 - (1) Set aside one purple top for Veterinary Diagnostic Services (VDS, only necessary for wolves) and one for the National Fish and Wildlife Forensic Laboratory (NFWFL, necessary for all species including wolves, dogs, and coyotes).
 - (a) Place these in separate bags, label, and refrigerate.
 - (2) Transfer all remaining blood in purple tops to cryovials and freeze for banking at UNM.

- (a) Label cryovials with species, studbook number, date, and “purple top.”
- iii. Ensure all blood tubes and vials are labeled with species, sex, studbook number, date, and contents for cryovials (“serum,” “red blood cells,” or “purple top”).
- iv. Blood is shipped to two different locations.
 - (1) One purple top for each animal (all canids) to Steve Fain.
 - (a) send the labeled purple tops triple bagged to:

Steve Fain
National Fish and Wildlife Forensic Laboratory
1490 E. Main Street
Ashland, OR 97520
 - (b) Include a written request for the following tests:
 - (i) Parentage (only if sample is from a wolf); provide studbook numbers for any wolves that might be the parents of the wolf from which the sample was obtained.
 - (ii) Confirmation that it is or is not a Mexican wolf.
 - (iii) Species identification.
 - (2) The remaining wolf samples will be sent to the University of New Mexico (UNM).
 - (a) One purple top and one cryovial of serum to Veterinary Diagnostic Services (VDS).
 - (i) Place one purple top and one cryovial of serum (at least 1 ml) in a bag.
 - (ii) Label the bag for each individual animal.
 - (iii) Mark bag “VDS” so that when it arrives at UNM Cheryl Parmenter will know where it needs to go.
 - (iv) When shipping blood for more than one wolf, separate samples for each animal into separate bags and place in one bag labeled VDS.
 - (b) The remaining samples go to Cheryl Parmenter to bank.
 - (i) The remaining “serum” and all other cryovials (“purple tops” and “red blood cells” go to Cheryl).
 - (ii) Put samples from individual wolves in separate bags and place all these bags in one labeled “Cheryl.”
 - (c) Put both bags labeled “VDS” and “Cheryl” into one bag. The samples are now triple bagged; this is standard for items considered a “bio hazard.”
 - (d) Any fecal samples that are collected go to VDS for a parasitology.
 - (e) Ship all samples to the following address:

Cheryl Parmenter
University of New Mexico
Department of Biology
167 Castetter Hall
Albuquerque, NM 87131
 - (f) Place contents into cooler or insulated shipper with ice packs (blue ice) and packaging material to protect the samples from breaking.
 - (g) Include copies of the capture sheets for each wolf in a separate bag so that they have records of these wolves for their files.
 - (h) Tape cooler shut with strapping tape to secure lid.

- (i) All blood will be shipped Federal Express Overnight.
- (j) If shipping cannot take place immediately samples should be preserved appropriately.
- (k) Purple tops for genetics must arrive at the lab within one week of being collected from the animal.
- (l) Ensure that blood will arrive the following day at the lab on a workday. Do not ship on Fridays, weekends, or holidays. Always call the labs to notify them blood is on the way to ensure someone is there to receive it.
- (3) Purple tops for CBC need to get to VDS in three days or less.
- (4) If not able to ship in this time frame, freeze blood extracted from purple tops in cryovials to be shipped at earliest opportunity for genetics to Steve Fain at Ashland lab.
- (5) When the serum is extracted from the red top tubes it can be frozen and banked or shipped at any time.

3. Storage.

- a. The only method for preserving blood at the Alpine Field Office is to freeze it.
 - i. Follow procedures described above.
 - ii. This should be used only for temporary purposes until other equipment or facilities are available at the Field Office.
 - iii. Blood from purple tops can be transferred into cryovials and frozen for genetics.
 - iv. Serum can be frozen in cryovials for future blood chemistry analysis.

Approvals:

The Mexican Wolf Blue Range Reintroduction Project Adaptive Management Oversight Committee approved this SOP on November 18, 2004.

References: None

Appendix A.

BLOOD COLLECTION FROM WILD CANIDS

Prepared by Marianna Roetto, DVM

Introduction

Blood collection from wild canids is a basic yet essential skill utilized frequently in wild canid research. The goals of successful blood collection are to:

1. Obtain an adequate sample for laboratory testing,
2. Collect a high quality sample,
3. Minimize handling stress to the animal, and
4. Minimize safety risks for the researcher

Equipment

Blood collection can be performed utilizing sterile syringes/needles or a vacutainer collection system

Syringe/needle collection

Advantages: Precise control of pressure applied during collection - prevents small veins from collapsing.

Disadvantages: Must transfer blood into collection tubes following sampling. Multiple samples, high volumes, require repeat venipuncture.

Vacutainer system

Advantages: Samples collected directly into sterile vials. Availability of various premeasured additives (anticoagulants) for specific laboratory tests. Ability to obtain multiple samples from single venipuncture.

Disadvantages: Vacuum pressure may collapse small veins.

Tourniquets

I prefer a quick release tourniquet strap.

Needles

Larger bore needles are preferred for blood collection as they reduce hemolysis (breakage of red blood cells) during collection. Hemolysis can alter many laboratory test results. At least a 20 gauge needle, or smaller gauge, should be used for blood collection (smaller gauge means larger diameter needle). Blood samples can be readily obtained from average size adult coyotes with a 20 gauge needle. Smaller animals may require the use of larger gauge needles. Generally, I prefer a 1 inch long needle for routine blood collection.

Collection methods:

The animal must be adequately immobilized for blood collection to reduce blood collection time, stress on the animal, and risk of injury to the researcher.

Blood can be collected from a variety of locations. The choice of collection site depends on the volume needed, the accessibility of the site, and the preferences of the collector. Common collection sites include the cephalic vein, lateral saphenous vein, jugular vein, and femoral artery. Cardiac collection is possible but the risk to the animal is high; therefore, I only recommend this site if the animal is to be euthanatized following collection. For coyotes, I prefer the cephalic vein with the jugular vein as a second choice.

Handy Hints for Successful Blood Collection

Apply tourniquet just above elbow for cephalic vein collection. Tourniquets should be just tight enough to distend the cephalic vein not tight enough to cause arterial occlusion or damage to the nerves. Other collection sites can best be occluded with digital pressure.

Vein identification can be increased by spraying the area with isopropyl alcohol which will raise the vein and flatten the hair in the vicinity.

Stabilize the vein with one hand to prevent rolling during venipuncture.

Always puncture the vein with the bevel of the needle up, with a firm motion. Slow entry into a vein will increase the likelihood of the vein rolling and an unsuccessful collection.

If blood flow stops during collection, try to rotate the needle slightly or advance and/or withdraw the needle slightly, parallel with the vein. Passive motion (gentle squeezing of the foot) may increase blood flow. If blood flow stops repeatedly, the vein is probably collapsing and you may have to use a syringe and needle.

If a hematoma (swelling) appears around the venipuncture site, it is best to withdraw the needle and select another venipuncture site. Although blood can frequently be collected with a hematoma present, the blood is usually of poor quality (hemolyzed) and may influence laboratory results.

If blood is transferred from a syringe to a sample vial, remove the needle from the syringe and the stopper from the vial, gently transfer blood down the side of the vial to minimize hemolysis, replace the stopper firmly.

After withdrawing the needle, apply firm pressure to the site until bleeding stops.